

RESEARCH ARTICLE

Long-term genetic monitoring of a translocated population of collared brown lemurs

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Abstract

Post-release monitoring is important to improve translocation success because it provides an opportunity to identify factors relevant to the survival of local populations. We studied a population of the endangered collared brown lemur (*Eulemur collaris*) translocated from a degraded forest fragment to a nearby littoral forest within the Mandena Conservation Area in southeast Madagascar from 2000–2011. We compared genetic surveys of mitochondrial and nuclear markers with the genetic profile of nearby populations to examine the dispersal capacity of the collared brown lemur. We also performed a landscape analysis to assess changes in connectivity between forest fragments. There was a fluctuating trend characterized by a phase of demographic and genetic stability shortly after translocation, followed by an increase in genetic diversity coinciding with a population decrease and a gradual recovery

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of initial conditions. These results demonstrated the ability of the collared brown lemur to disperse through unfavorable landscapes and to recover after translocation. Our study revealed the importance of monitoring translocated populations over time using a multidisciplinary approach.

KEYWORDS

Eulemur collaris, genetic diversity, littoral forest, Madagascar, translocation

Habitat loss due to land use change is the main threat to species persistence (Dale et al. 1994, Dolman and Sutherland 1995, Brooks et al. 2002). While initial degradation of a pristine environment can generate habitat heterogeneity and promote increased biodiversity (Fahrig 2017), severe habitat loss is often linked to landscape fragmentation and increased isolation of patches of suitable habitat, with negative consequences for biodiversity (Wiegand et al. 2005, Fletcher et al. 2018). Adverse effects include contraction of population size (Andren 1994), resource depletion (Gabriel et al. 2018), spread of diseases (Nunn and Altizer 2006), and loss of genetic diversity (Lino et al. 2019, González et al. 2020). Conservation actions involving animal translocation are increasingly common to contain local extirpation (Strum 2005, Fitzgerald et al. 2015); however, these management solutions are risky and recent estimates indicate that only about half of them are successful (Bubac et al. 2019). In addition to the habitat of the release area, post-release monitoring and management are also important to the success of translocations (Fischer and Lindenmeier 2000, Armstrong and Seddon 2008). Monitoring translocation success can help determine the primary causes of population fluctuations and enable rapid conservation actions (Bubac et al. 2019, Resende et al. 2020). Defining the success of an animal translocation is difficult because the criteria followed in various studies are discordant (Morris et al. 2021). There is a consensus that a translocation can be considered successful when it leads to the creation of a viable self-sustaining population, but interpretations may vary depending on the reasons why the animals were translocated (Seddon 2010, International Union for Conservation of Nature/Species Survival Commission 2013).

Madagascar's lemurs are among the most threatened mammal groups in the world (Schwitzer et al. 2014). Habitat degradation affects their ecology (Lehman and Rajaonson 2006, Irwin 2007, Donati et al. 2011, Eppley et al. 2020, Gould et al. 2020), genetics (Radespiel et al. 2008, Sgarlata et al. 2018), and abundance (Ganzhorn et al. 2003, Dunham 2008, Steffens and Lehman 2018). The endangered collared brown lemur (*Eulemur collaris*) is the largest primate in the littoral forest of southeast Madagascar, where it has an ecological role as a seed disperser (Bollen et al. 2004, Andriaholinirina et al. 2014). The littoral forest represents one of the most diverse and degraded ecosystems on the island (Bollen and Donati 2006, Callmänder et al. 2007), with nearly 90% of its original extent converted to non-forested land cover types in the last century (Consiglio et al. 2006). In 2000 and 2001, a small population of collared brown lemurs was translocated from severely degraded forest fragments to the nearby Mandena Protected Area (MND), where the species was previously extirpated because of illegal hunting (Donati et al. 2007, 2020b). The recent protected status, the presence of a research station, and the positive response of surrounding villages to MND were considered safeguards to prevent the initial cause of extirpation. The rescue operation was promoted and funded by MND's manager (Quebec Iron and Titanium Madagascar Minerals) with support from the Madagascar Fauna Group and representatives from Madagascar National Parks, Ministère des Eaux et Forêts, and World Wildlife Fund. Subsequent post-release monitoring of these translocated lemurs revealed a high degree of ecological flexibility, displayed through a reduction in group size (Donati et al. 2011), expansion of range areas (Campera et al. 2014), variation in activity pattern (Donati et al. 2016), and shift in diet toward exotic plant species during the initial phase (Donati et al. 2020b). While ecological flexibility is a trait that favors lemur persistence

in degraded habitats (Irwin 2007, Eppley et al. 2020), dispersal ability is important to their survival (Steffens and Lehman 2018, Gould and Cowen 2020). Collared brown lemurs do not disperse efficiently across fragmented forests (Ranaivoarisoa et al. 2010, Bertoncini et al. 2017), but recent observations indicate that these lemurs can cross approximately 1.5 km of open savanna (Hyde-Roberts et al. 2020). Detailed investigations of the effect of dispersal on the long-term persistence of locally isolated populations are still rare (Arroyo-Rodríguez et al. 2013).

We investigated the genetics of a translocated population of collared brown lemurs from 2000 to 2011. Genetic monitoring of this population provided an opportunity to examine dispersal ability and determine connectivity with neighboring fragments. We supplemented genetic information with demographic data to identify factors that threatened the survival of the population examined.

STUDY AREA

We conducted this work in 2000–2011 in MND (Ganzhorn et al. 2007), a littoral forest located about 10 km northwest of Fort Dauphin (24°57'S, 47°0'E) in southeast Madagascar (Figure 1). Littoral forest grows on sandy soils and occurs within 2–3 km of the coast, at an altitude of 0–20 m (Dumetz 1999). This area consists of 2 forest fragments (with an area of ~230 ha) connected by a swamp, but it can be considered as a single unit because lemurs regularly cross the swamp. This region is characterized by a humid tropical climate with no distinct dry season and an average annual rainfall of about 2,000 mm (Vincelette et al. 2007). Detailed information on MND's fauna and flora has been previously published (Ganzhorn et al. 2007).

METHODS

Census and sampling

In 2000–2001, we translocated 28 collared brown lemurs (all those present) from a forest fragment under anthropogenic pressure due to charcoal production in the MND (Donati et al. 2007). The source and target forests had similar sizes (~260 ha, ~230 ha) and similar floristic composition, and were about 2 km apart (Donati et al. 2007). We conducted censuses in 2000, 2004, 2008, 2009, 2010, 2011, 2014, and 2017. Twenty observers were spaced at regular intervals and traversed the entire forest area (Donati et al. 2007). We completed the surveys in a single day and communicated with radios to minimize the risk of double counting. We also determined sex of each animal observed. The sexual dichromatism facilitated sex identification (Donati et al. 2020a).

We collected ear clips and feces from 89 animals. We collected 4 ear clips in 2000–2001 and 11 in 2004, and collected 6 fecal samples in 2008 and 13 in 2011. We collected 4 fecal samples in 2008 at Nahampoana Reserve (NHM; 24°58'S, 46°58'E), located about 1 km southwest of MND. We sampled 2 areas of the Tsitongambarika protected lowland forest (TGK); we collected 7 fecal samples from TGK1 (24°53'S, 46°58'E) and 9 from TGK3 (24°33'S, 47°08'E). We sampled 2 forest fragments in the Sainte Luce conservation area (S); we obtained 20 fecal samples from S9 (24°46'S, 47°10'E) and 5 from S17 (24°48'S, 47°09'E). We placed each fecal sample in a 96% ethanol solution within minutes after defecation and stored samples at 4°C until DNA extraction. The DNA data of MND in 2011, TGK1, TGK3, S9, and S17 are derived from Bertoncini et al. (2017).

Landscape analysis

To understand how connectivity among fragments changed between 2005 and 2015, we estimated the distance of least resistance between forest areas using GRAPHAB 2.4 (Foltête et al. 2012) and ArcGIS 10.4 software

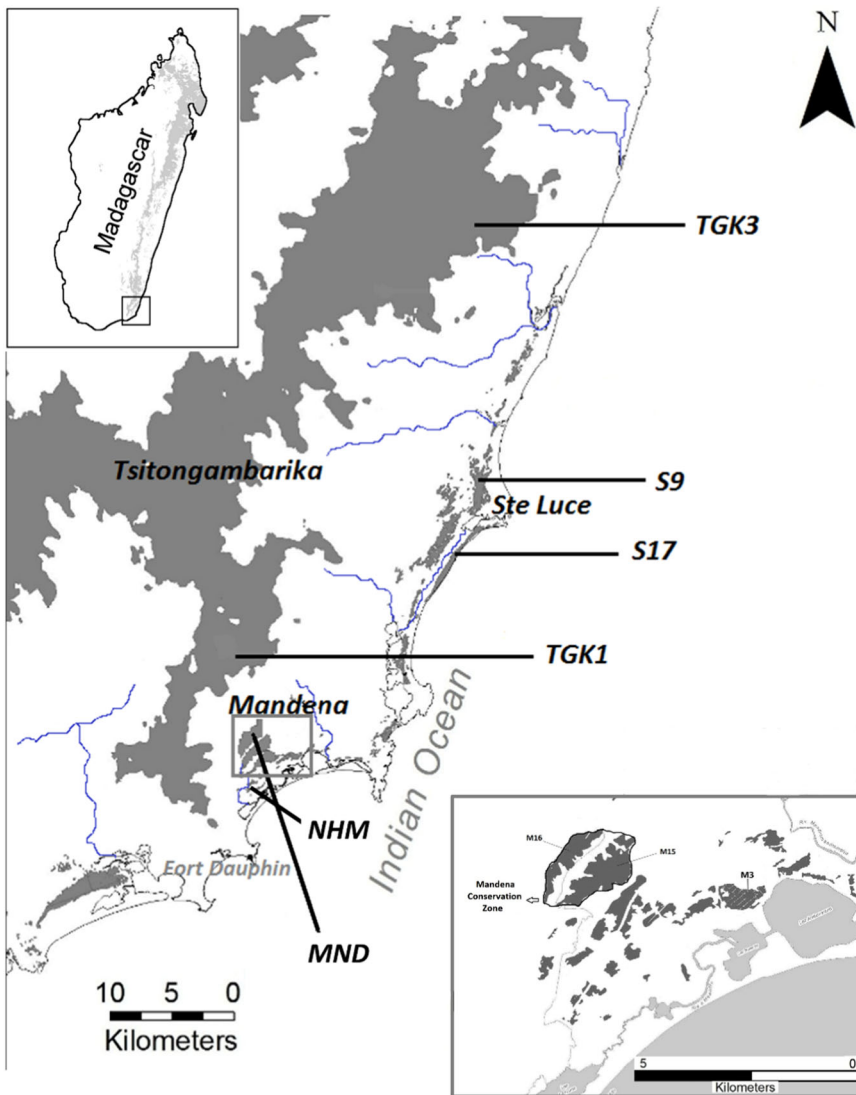


FIGURE 1 Location of the study sites of the brown collared lemur, in southeast Madagascar, 2000–2011. Sites included Tsitongambarika 1 (TGK1), Tsitongambarika 3 (TGK3), Mandena (MND), Sainte Luce 9 (S9), Sainte Luce 17 (S17), and Nahampoana (NHM).

(Esri, Redlands, CA, USA). We downloaded 2 satellite images from 2005 and 2015, from Google Earth Pro to create a raster. We used the image classification function in ArcGIS with an unsupervised algorithm to divide the map into 5 clusters based on the level of greenness of the area: 1) forest, 2) degraded forest, 3) bush, 4) savanna, and 5) open area. We generated the least resistance links between forest areas with the minimum spanning tree algorithm in GRAPHAB, using different resistance values for each habitat (forest = 1, degraded forest = 5, shrub = 80, savanna = 95, open area = 100; Foltête et al. 2012; Figures S1, S2, available in Supporting Information). We specified the resistance values taking into consideration the locomotion style (predominantly arboreal) of collared brown lemurs (Donati et al. 2020a). We then exported the GRAPHAB links to ArcGIS and estimated the minimum distance and the extent of open areas between forest patches.

DNA characterization and quality control

We followed manufacturer instructions to extract genomic DNA from tissue samples using the EasySpin® Extraction Kit (Qiagen, Hilden, Germany), and from fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen). The workflow for mitochondrial and nuclear DNA genotyping followed Bertoncini et al. (2017).

As for the mitochondrial DNA, we sequenced 320 base pairs of the control region with the ABI 310 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA) using primers obtained from Bertoncini et al. (2017). We used the mitochondrial sequence Genbank ID:AF257980 as reference (RS) following Wyner et al. (2002) and Bertoncini et al. (2017). We assured the reliability of mitochondrial sequencing by replicates performed on 54% of *de novo* extracted DNAs (15 of 28). We uploaded the mitochondrial sequences to GenBank (accession numbers MH029595–MH029626).

We selected 8 autosomal short tandem repeat (STR) loci from the literature (Jekielek and Strobeck 1998, Tokiniana et al. 2009, Ranaivoarisoa et al. 2010) and redesigned primers (Bertoncini et al. 2017) to obtain shorter amplicons, a strategy employed to maximize polymerase chain reaction (PCR) yield with low quality DNA. We analyzed amplification products on the ABI 310 Genetic Analyzer. We applied the stepwise system derived from Frantz et al. (2003) and adopted by Bertoncini et al. (2017) to ensure reliability of the microsatellite genotyping. We validated each PCR run with 3 negative controls. We discarded any genotype that did not pass this multi-step check. We employed Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004) to assess the presence of non-amplified alleles due to mutations at primer sites (i.e., null alleles), the preferential amplification of short alleles (i.e., large alleles dropout), and the occurrence of slippage during the PCR amplification (i.e., stuttering). To ensure that the set of microsatellite loci could reliably distinguish unique genotypes, we calculated the probability of identity (Paetkau and Strobeck 1994) and the probability of identity between siblings (Waits et al. 2001) with GenAlEx 6.5 (Peakall and Smouse 2012).

Statistical analysis

We estimated standard indices of genetic diversity to characterize changes for the population of MND during the post-release monitoring. We estimated the unbiased index of genetic diversity (h ; Nei 1987) with Arlequin version 3.5.1.2. (Excoffier and Lischer 2010). We employed HP-Rare version 1.1 (Kalinowski 2005) to calculate the number of alleles private to each population ($Pr_{-}al$), and the standardized measure of allelic diversity (Ar). We used the web-based GENEPOP (Raymond and Rousset 1995, Rousset 2008) to estimate the number of effective migrants per generation (N_m) with the private alleles method of Barton and Slatkin (1986). We used the same platform to verify the independence of allele distribution in various sampling years by adopting the Fisher's exact probability test (Fisher 1934). We used FSTAT 2.9.3.2 (Goudet 2002) to estimate the pairwise relatedness (r ; Hamilton 1971) for all the sampling groups.

We used the likelihood-based software GeneClass2 (Piry et al. 2004) to identify first-generation migrants using nuclear multi-locus data. We conducted this analysis using the statistical criterion L_{home}/L_{max} and the Bayesian approach developed by Rannala and Mountain (1997), and we estimated the P -value by re-sampling 1,000 individuals with the algorithm developed by Paetkau et al. (2004) with the threshold for type I error set at 0.01.

We used STRUCTURE 2.3.4 (Pritchard et al. 2000) with an admixture model to identify the main genetic clusters. We set the burn-in at 1,000 followed by 10,000 Markov chain Monte Carlo simulations. We determined the optimal number of genetic clusters (K) with the method of Evanno et al. (2005). We assessed the consistency of results performing 10 independent runs for each value of K . We employed DISTRUCT 1.1 (Rosenberg 2004) to display the results.

We estimated the effective population size (N_e) following the stepwise mutation model (SMM) employing the formula $\theta = 4N_e\mu$ (Ohta and Kimura 1973) and we retrieved the mutation rate (μ) for microsatellite markers from

Balaresque et al. (2009; 0.0021 mutations/locus/generation). We computed the Watterson estimator (θ ; stepwise mutation model; Kimura and Ohta 1978) with Arlequin version 3.5.1.2. (Excoffier and Lischer 2010).

As we sampled only a fraction of the number of individuals of MND in 2000–2001 and 2004, 2 mutually exclusive hypotheses arise following the detection of a new haplotype in 2008: 1) the population was dimorphic in 2000–2001 and 2004, but the second haplotype (RS) remained undetected in 2000–2004 because of incomplete sampling, or 2) gene flow occurred between 2004 and 2008. We computed the probability of the first hypothesis following the theory of probability of combined events. The reciprocal of such value represents the probability of the second hypothesis. Given $N = a + b$, where N is the number of elements, a is the number of elements of type α , and b is the number of elements of type β , and given n = examined elements, with $n \leq N$, the probability P that n elements belong to the type α is given by the ratio of favorable outcomes to the number of possible outcomes.

Favorable outcomes: $a! / [(a - n)! \times n!]$

Possible outcomes: $N! / [(N - n)! \times n!]$

$P: \{a! / [(a - n)! \times n!]\} / \{N! / [(N - n)! \times n!]\}$

RESULTS

Demography and landscape change

There was a small decrease in population size between 2000 (28) and 2004 (25; Table 1, Figure S3, available in Supporting Information), followed by a decline between 2004 and 2008 (8) and a recovery in 2011 (17) and subsequent years. Census size and genetic diversity (h) showed an inverse relationship for both mitochondrial ($r = -0.86$, $P = 0.14$) and nuclear ($r = -0.93$, $P = 0.07$) markers. Although census sizes were not recorded for 2012, 2013, 2015, and 2016 and the exact progression cannot be reconstructed, the data revealed a consistent population increase from 2008 to 2017 (Table 1; Figure S3).

The distances of least resistance between forests were shorter in 2005 than in 2015, and deforested areas were larger in 2015 than in 2005 (Figure 2). Deforested areas between S17 and MND extended over 0.5 km in 2005, which increased to 1.1 km in 2015. The deforested areas between TKG1 and MND extended over 0.6 km in 2005 and 0.7 km in 2015. The alternative route between TKG1 and MND was 9.3 km in 2005 and 15.3 km in 2015.

Mitochondrial DNA haplotyping

All replicated mtDNA sequences matched the original sequences. Micro-checker revealed no evidence of null alleles, large allelic loss, or stuttering. The probability of identity and the probability of identity between siblings revealed that 2 different individuals could be confidently distinguished using a minimum of 3 and 7 loci, respectively (Figure S4, available in Supporting Information), indicating that the risk of ambiguous assignment of an individual is minimal. All DNA samples produced different microsatellite profiles, except for samples 19 F (2000 survey) and M4-A_14F (2004 survey; Table S1). Although it is possible that 19 F and M4-A_14F represent the same individual, this possibility does not invalidate our observations because the present work monitors changes in diversity of the original population. The microsatellite profiles from NHM did not meet quality standards and we did not consider them in further analysis.

We provided mitochondrial sequences for 11 of 28 of the individuals in 2000, 11 of 25 in 2004, 6 of 8 in 2008, and 13 of 17 in 2011. The MND population was monomorphic in each sampling year (HT1 was the only haplotype detected), except in 2008, when we found 2 haplotypes, HT1 and RS ($h = 0.5$). The HT1 haplotype was ubiquitous in

TABLE 1 Genetic diversity of the collared brown lemur in southeast Madagascar estimated at mitochondrial (control region) and nuclear (8 autosomal short tandem repeat [STR] loci) DNA, during 2000–2013. Sites included Nahampoana (NHM), Mandena (MND), Tsitongambarika 1 (TGK1), Tsitongambarika 3 (TGK3), Sainte Luce 9 (S9), and Sainte Luce 17 (S17). We present the number of animals (*n*), haplotype richness (*Hr*), unbiased genetic diversity (*h*; Nei 1987), rarefied allelic richness (*Ar*), fixation index (*Fis*), number of different alleles (*k*), number of private alleles (*Pr-al*), Watterson estimator (θ ; Ohta and Kimura 1973), and effective population size (*Ne*); some values are not determined (ND).

		Site									
		NHM	MND			TGK 1		TGK 3	S 9	S 17	
Forest type		littoral	littoral	littoral	littoral	littoral	lowland	lowland	littoral	littoral	
Geographic coordinates		24°58'S	24°57'S	24°57'S	24°57'S	24°57'S	24°53'S	24°33'S	24°46'S	24°48'S	
		46°58'E	47°0'E	47°0'E	47°0'E	47°0'E	46°58'E	47°08'E	47°10'E	47°09'E	
Sampling year		2008	2000	2004	2008	2011	2011	2012	2013	2013	
Census size		ND	28	25	8	17	ND	ND	ND	ND	
Mitochondrial DNA											
<i>n</i>		4	11	11	6	13	7	8	11	4	
<i>Hr</i>		2	1	1	2	1	1	2	1	1	
<i>h</i> ± SD		0.500 ± 0.265	0	0	0.533 ± 0.172	0	0	0.571 ± 0.095	0	0	
Nuclear DNA											
<i>n</i>		ND	14	10	5	11	4	9	20	5	
<i>Ar</i>		ND	1.84	1.79	2.33	1.73	2.04	2.07	2.01	1.91	
<i>Fis</i>		ND	0.029 ± 0.076	−0.025 ± 0.098	0.055 ± 0.153	0.153 ± 0.156	0.760 ± 0.111	−0.272 ± 0.105	0.376 ± 0.139	0.022 ± 0.103	
<i>k</i>		ND	17	14	19	20	19	23	25	20	
<i>Pr-al</i> (%)		ND	0 (0%)	0 (0%)	2 (10%)	0 (0%)	3 (16%)	3 (13%)	5 (20%)	3 (15%)	
<i>h</i> ± SD		ND	0.327 ± 0.201	0.339 ± 0.21	0.439 ± 0.276	0.349 ± 0.213	0.595 ± 0.381	0.533 ± 0.309	0.423 ± 0.251	0.425 ± 0.272	
θ		ND	1.771	1.73	1.583	1.63	1.507	1.509	1.5	1.517	
<i>Ne</i>		ND	210	206	188	194	ND	ND	ND	ND	

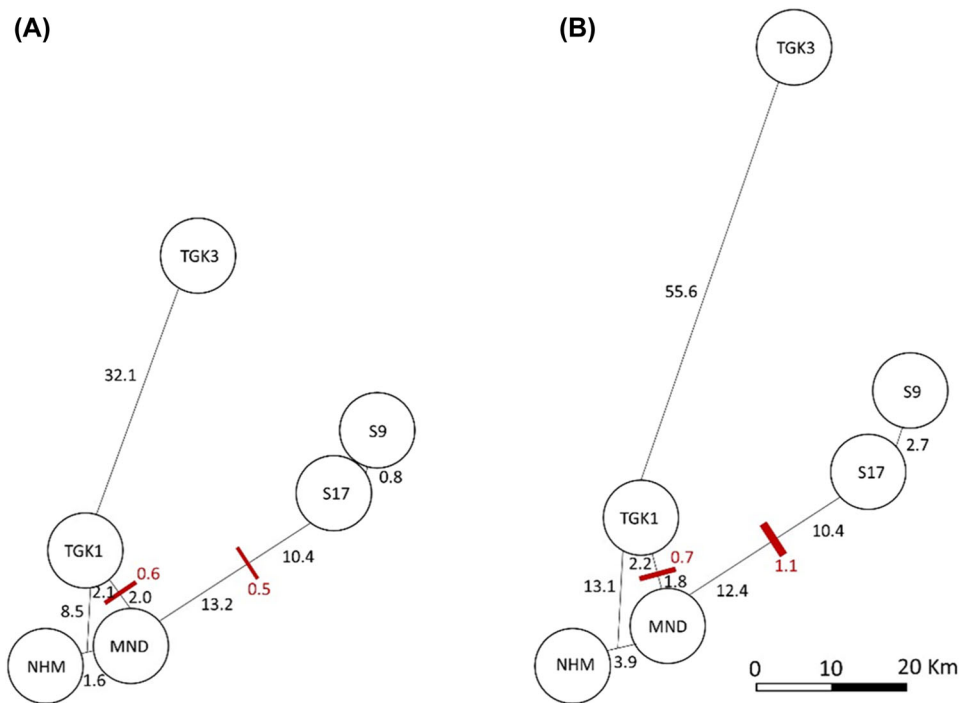


FIGURE 2 Minimum distance of least resistance (thin lines) and open areas (thick lines) for the collared brown lemur populations in southeast Madagascar in 2005 (A) and 2015 (B). The length of the segment is proportional to the minimum distance (km) of least resistance created via GRAPHAB 2.4. The thickness of the lines indicating open areas is proportional to the extent of the open areas (km). Sites included Tsitongambarika 1 (TGK1), Tsitongambarika 3 (TGK3), Mandena (MND), Sainte Luce 9 (S9), Sainte Luce 17 (S17), and Nahampoana (NHM).

populations surrounding MND (NHM, S9, S17, TGK1, and TGK3), while RS was found only in NHM ($h = 0.533$) and HT2 only in TGK3 ($h = 0.571$; Table S1).

Application of the theory of probability of combined events to the present case study (Table S3, available in Supporting Information) revealed that the probability that each of the haplotypes sampled in 2000–2001 represented HT1, despite the presence of RS, is 0.36. Similarly, the probability that each of the haplotypes sampled in 2004 represented HT1, despite the presence of RS, is 0.30. The combined probability of the first and second events is 0.11 and represents the probability that the population was dimorphic in 2000–2001 and 2004, but RS was undetected during those sampling years. The reciprocal of this value is 0.89 and represents the probability that gene flow occurred between 2004 and 2008.

Microsatellite genotyping

We provided nuclear microsatellite coverage for 14 of 28 of the individuals in 2000, 10 of 25 in 2004, 5 of 8 in 2008, and 11 of 17 in 2011. Compared with the founder gene pool, 5 new alleles appeared in 2008 and 2 appeared in 2011 (Table S1). Furthermore, a female (MC2_f) carried 4 new alleles (1 in homozygosity) in 2008 and a male (1M) carried 2 new alleles (both in homozygosity) in 2011, which suggests that these 2 individuals were migrants.

Parameters such as A_r , h , and Pr -al consistently had the lowest values of genetic diversity for 2000–2001 and 2004, and the highest in 2008. We observed a decrease in diversity in 2011 (Table 1). The bivariate graph showing

the kinship (r) of the temporal subsets of the MND population with the gene pools of the other 4 areas as a function of the number of migrants (N_m) revealed a higher kinship of the MND population in 2008 with neighboring populations, particularly with S17 (Figure 3). The STRUCTURE analysis based on 4 genetic clusters confirmed the presence of new MND genotypes in 2008 and 2011 (Figure 4). At least 2 animals, 1 M and M2Cf, revealed similar

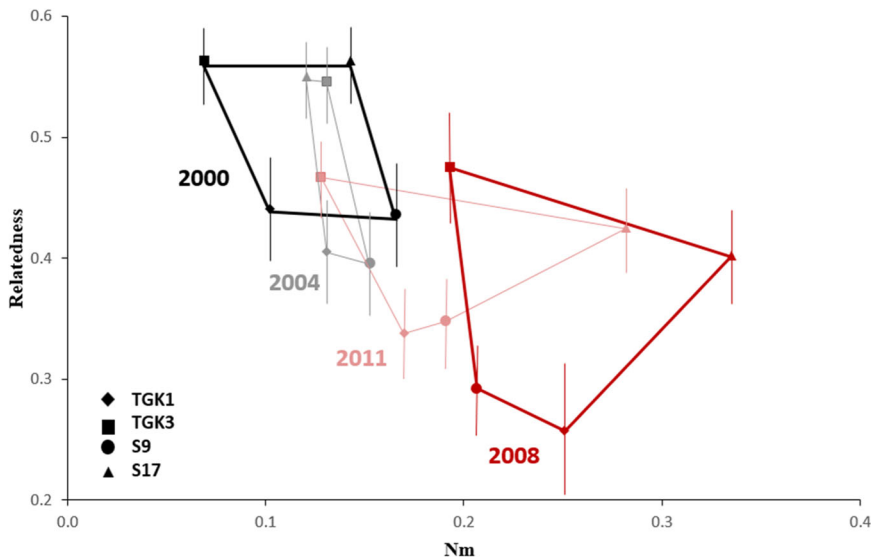


FIGURE 3 Scatter plot of the pairwise relatedness and number of migrants (N_m) between the collared brown lemur populations at Mandena (4 sampling years) and at the other 4 sampling sites in southeast Madagascar, 2000–2011. Sites included Tsitongambarika 1 (TGK1), Tsitongambarika 3 (TGK3), Sainte Luce 9 (S9), and Sainte Luce 17 (S17).

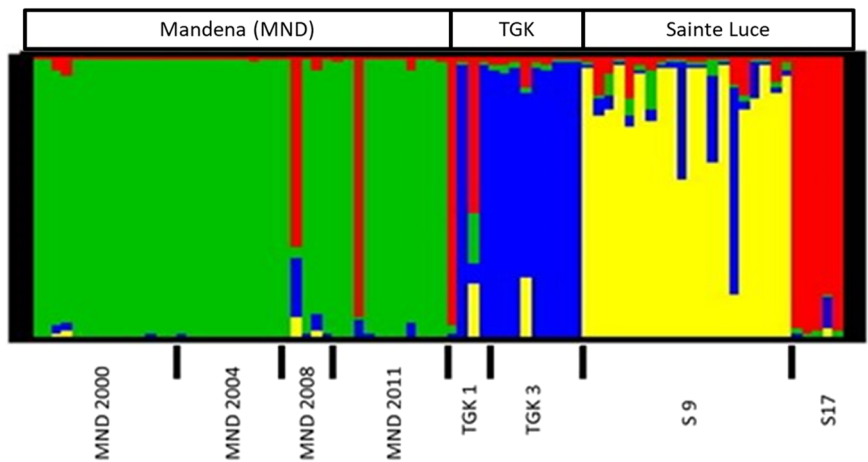


FIGURE 4 STRUCTURE analysis (Pritchard et al. 2000) for 5 populations of the collared brown lemur at 8 microsatellite loci. We analyzed the population from Mandena, Madagascar, in 4 different years (2000, 2004, 2008, 2011) with 4 genetic clusters (K). Sites included Mandena (MND; 2000: $n = 14$; 2004: $n = 10$; 2008: $n = 5$; 2011: $n = 11$), Tsitongambarika 1 (TGK1; $n = 4$), Tsitongambarika 3 (TGK3; $n = 9$), Sainte Luce 9 (S9; $n = 20$), and Sainte Luce 17 (S17; $n = 5$).

genetic composition to individuals from TKG1 and S17 (Figure 4; Table S1). Geneclass2 analysis (Table S2, available in Supporting Information) supported individual 1 M as the migrant ($P < 0.05$) and S17 as the most likely source of migration.

The initial N_e estimated by θ was 210 in 2000–2001, 206 in 2004, 188 in 2008, and 194 in 2011 (Table 1), while remaining below the conventional threshold of long-term extinction threat ($N_e = 500$; Franklin and Frankham 1998).

DISCUSSION

Long-term monitoring data are important for determining the success of primate translocations (Beck 2016, 2019) and for understanding the role of ecological and random factors (Silver and Marsh 2003, Strum 2005, Cristóbal-Azkarate and Dunn 2013, Bubac et al. 2019, Gould et al. 2020). The present translocation involved the movement of several groups of wild lemurs to a recently protected but partially degraded release site (Donati et al. 2007, Rabenantoandro et al. 2007). Our post-release monitoring illustrates 2 main findings. First, we identified several immigrating animals, indicating that collared brown lemurs can disperse over significant distances in non-optimal habitats. Second, despite fluctuating census size and genetic diversity over the years, the lemur population showed resilience and overall population growth. Both results suggest that collared brown lemurs, despite their arboreal habit and habitat requirements, are flexible enough to cope with translocations and repopulate fragmented regions over time. This flexibility is in line with previous behavioral and ecological studies on the same species (Donati et al. 2011, 2016, 2020b; Balestri et al. 2014; Campera et al. 2014; Hyde-Roberts et al. 2020) and on other species of brown lemurs (Sato et al. 2016, Steffens and Lehman 2018).

Metapopulation theory is widely used to describe spatial patterns of diversity in fragmented habitats (Ovaskainen and Saastamoinen 2018), enabling predictions of extirpation risk (Zeigler et al. 2013), but the theory has been rarely applied to lemurs (Steffens and Lehman 2018). Such an approach requires detailed spatial information on fragmented habitat and knowledge of the dispersal capacity of the species examined (Ovaskainen and Saastamoinen 2018). Although dispersal ability is fundamental to understanding the biology of a species for effective conservation (Santini et al. 2013), dispersal has been estimated only for a few lemur species (Quéméré et al. 2010, Schliehe-Diecks et al. 2012, Baden et al. 2014). This lack of information reflects the difficulty of data collection due to the rarity of dispersal events (Nathan et al. 2003). The present work identified several dispersal events, supporting previous reports that brown lemurs can use riparian forests, non-forested areas, or both during their dispersal (Donati et al. 2007, Steffens and Lehman 2018, Hyde-Roberts et al. 2020). This ecological flexibility is consistent with previous data indicating that collared brown lemurs adapt group size, activity pattern, and range-shifting behavior to cope with habitat fragmentation (Donati et al. 2011, 2016, 2020b; Campera et al. 2014). Our genetic analysis shows a general concordance between the mitochondrial and nuclear data, indicating an increase in diversity in MND in 2008 compared to the gene pool of the founding groups (surveys 2000–2001, 2004), followed by a decrease 3 years later (2011 survey). This fluctuating trend can be explained by introgression and loss of genetic elements through stochastic or unpredictable mechanisms such as drift and gene flow (Gilpin and Soulé 1986, Lacy 1987, Frankham 1998). The presence of a new mitochondrial haplotype in 2008 suggested that a female (MC1_f) and a male (MC2_m) probably migrated to MND between 2004 and 2008. The new haplotype may have been present and undetected in the population as early as 2000–2001, but this hypothesis has a low probability (0.11). Unfortunately, the microsatellite profile of the individuals carrying this new haplotype could not be determined because of the poor quality of the DNA extracts.

We found new microsatellite alleles in homozygosity in 2 individuals, a female (MC2_f) in 2008 and a male (1M) in 2011. Because homozygous rare alleles are not typically found in populations at equilibrium (Hardy 1908, Weinberg 1908), this occurrence provides additional evidence of gene flow. Comparison with neighboring populations indicated the 2 sites of TKG1 and S17 as potential origins of dispersal. The first of these locations,

TGK1, is part of the Tsitongambarika Protected Area, a large continuous lowland rainforest that has received official protection since 2008 (Birdlife International 2011, Schwitzer et al. 2014). The area is estimated to contain one of the largest remaining populations of collared brown lemurs (Campera et al. 2019). The shortest distance of least resistance estimated by our analysis between MND and TGK1 was about 4.7 km in 2005 but included deforested areas and the crossing of the RN12 national road. The alternative path of least resistance between the 2 areas generated by our analysis measured 9.3 km in 2005. Although these estimated distances are considerable for arboreal mammals (Prugh et al. 2008, Bannar-Martin 2014), collared brown lemurs have substantial ecological flexibility (Donati et al. 2011, 2020b). This trait may have facilitated riparian forest use and dispersal into the savanna. Our hypothesis is consistent with the observation in 2001 of some MND individuals crossing about 2 km of savanna in an attempt to return to their original fragments several months after their translocation (Donati et al. 2007). Another recent observation of dispersal across about 1.5 km of open grassland indicates that this species can disperse over relatively large distances in fragmented landscapes (Hyde-Roberts et al. 2020).

It is much more complicated to explain dispersal to MND from S17, a forest fragment included in the protected area of Sainte Luce. Mandena and S17 are separated by natural and anthropogenic barriers such as savanna, swamps, a river, and an active mining site. The possibility of a lemur moving through the few patches of riparian forest between the 2 areas is remote, considering that we estimated a distance of least resistance of about 24 km. An alternative hypothesis, supported by the mitochondrial results, is that this male moved to MND from nearby NHM, a small private reserve only 0.8 km southwest of MND, known to contain several collared brown lemurs. Because NHM is a small, non-natural forest, it is possible that some animals were moved there from other forests in the region. This hypothesis should be considered very plausible, given the growing popularity of free-ranging lemur parks as a tourist attraction and the growing eco-tourism market in the nearby city of Tôlanaro (Reuter et al. 2016, 2017).

The census size of the translocated population in MND displayed a fluctuating trend, with an inverse association with genetic diversity. Census size showed the lowest value in 2008, but we observed a rebound effect in 2011 that lasted until 2017. Although some instances of hunting by humans contributed to the initial population decline (Borgerson et al. 2016, Brook et al. 2019), improved conservation management of the forest (Ganzhorn et al. 2007) and the regular presence of research assistants in the area have likely deterred hunting these animals. We cannot rule out that resource scarcity may have played a role in the population decline observed in 2008, but it is plausible that predation was a more serious problem. Since 2003, several cases of killing by fossas (*Cryptoprocta ferox*), the largest carnivore in the area and a predator of lemurs (Donati et al. 2007, Hawkins and Racey 2008), have been recorded. Predation is a major factor in the failure of translocation projects (Yeager and Silver 1999, Fischer and Lindenmayer 2000), and reintroduced animals usually require specific anti-predator training programs (Griffin et al. 2000, Britt et al. 2001, Britt and Lambana 2003, Cheyne 2009, Guy and Curnoe 2013). Because the collared brown lemurs in this study came from a wild population and were already exposed to natural predation, it is unclear why several animals were killed by fossas. One possible hypothesis is that the relocated lemurs behaved naively with fossas because they came from another fragment where this predator was only sporadically present based on previous reports (Lewis Environmental Consultants 1992). The population increase observed after 2008 was accompanied by an overall increase in the estimated minimum distances of least resistance between fragments (Figure 4), a scenario that may have hindered the dispersal of fossas into the MND forest and encouraged the growth of the resident lemur groups.

A comparison between census size and genetic estimation of N_e provided further support for the dispersal of this species through non-optimal habitats. The effect of even occasional population contractions heavily affects estimates of N_e (Gilpin 1991). Thus, a fluctuating population, as generally observed in many mammal species, produces a low effective size when compared to census size. Our results, however, indicate that the N_e of the translocated population is about 10 times larger than the census size. This seemingly counterintuitive result can be explained by considering that genetic estimates of N_e are based on the number of segregating sites under

mutation-drift equilibrium in closed populations (Watterson's θ estimator; Ohta and Kimura 1973). Because these assumptions can be violated in natural populations, 2 scenarios are compatible with our results. A recent introgression of genetic variants in 2008 may have produced a stronger increase in N_e than census size (Baalsrud et al. 2014). Alternatively, fragmentation of a larger ancestral population (Bertoncini et al. 2017) could have produced mutation-drift disequilibrium resulting in high N_e estimates.

RESEARCH IMPLICATIONS

Although stochastic events, such as deaths and migrations, have a predictable strong effect on small populations, the experience of this translocation illustrates that brown lemurs tolerate relocations and adapt (to some extent) to relatively degraded forest fragments (Donati et al. 2020b). Our work suggests that a positive perspective can be maintained if rescue translocations become necessary because of the ongoing mining in the littoral forest region (Ganzhorn et al. 2007), or to re-stock existing populations (Bertoncini et al. 2017). More broadly, although habitat differences should be considered, our work offers insights for the relocation of brown lemurs in other fragmented areas of Madagascar. Because brown lemurs are important seed dispersers in Malagasy forests (Bollen et al. 2004), this may facilitate forest diversity maintenance or passive ecological restoration of degraded habitats (Steffens 2020, Konersmann et al. 2021). Finally, the relative isolation of MND and the duration of our post-monitoring offered an opportunity to test the ability of this species to disperse across non-forested areas. The evidence of immigrating animals demonstrates the importance of habitat connectivity to re-establish gene flow and avoid local extirpations (Steffens and Lehman 2018, Eppley et al. 2020).

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

ETHICS STATEMENT

The work was conducted with the authorization of the Commission Tripartite of the Direction des Eaux et Forêts de Madagascar (Autorisation de recherche n. 29/11/MEF/SG/DGF/DCB.SAP/SCB du 20/01/11).

DATA AVAILABILITY STATEMENT

The mitochondrial sequences are uploaded to GenBank (Accession numbers: MH029595–MH029626). Microsatellite data are available in the Supporting information.

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